Constituents of the Vietnamese Medicinal Plant *Streptocaulon juventas* and Their Antiproliferative Activity against the Human HT-1080 Fibrosarcoma Cell Line

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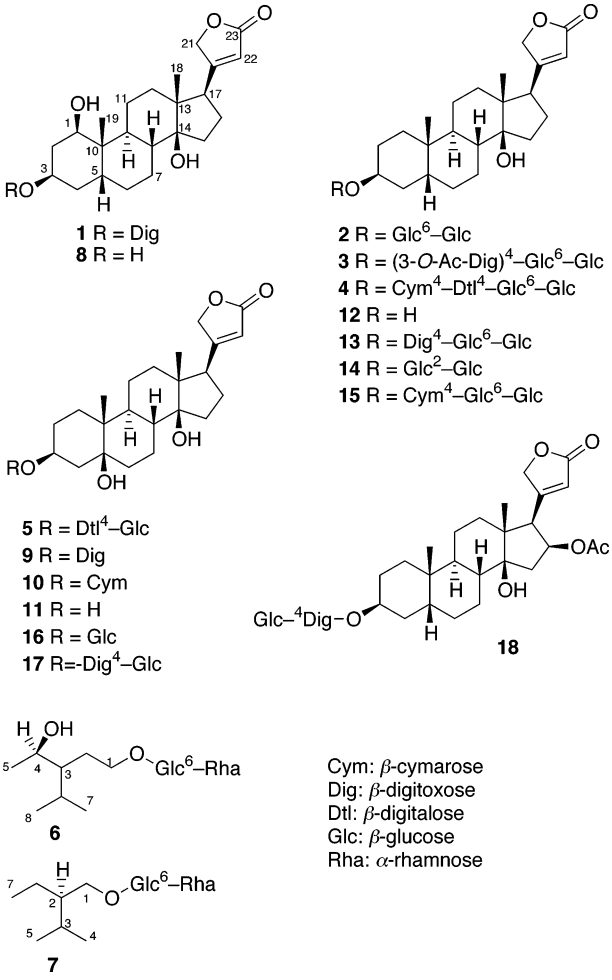
The methanolic extract of roots of *Streptocaulon juventas*, having shown strong antiproliferative activity against the highly metastatic human HT-1080 fibrosarcoma cell line, was subjected to activity-guided isolation to yield 16 cardenolides including five new ones, acovenosigenin A 3-*O*-*β*-digitoxopyranoside (**1**), digitoxigenin gentiobioside (**2**), digitoxigenin 3-*O*-[*O*-*β*-glucopyranosyl-(1→6)-*O*-*β*-glucopyranosyl-(1→4)-3-*O*-acetyl-*β*-digitoxopyranoside] (**3**), digitoxigenin 3-*O*-[*O*-*β*-glucopyranosyl-(1→6)-*O*-*β*-glucopyranosyl-(1→4)-*O*-*β*-digitalopyranosyl-(1→4)-*β*-cymaropyranoside] (**4**), and periplogenin 3-*O*-(4-*O*-*β*-glucopyranosyl-*β*-digitalopyranoside) (**5**), and two new hemiterpenoids, (4*R*)-4-hydroxy-3-isopropylpentyl *β*-rutinoside (**6**) and (*R*)-2-ethyl-3-methylbutyl *β*-rutinoside (**7**), together with two known phenylpropanoids and a known phenylethanoid. The isolated cardenolides strongly inhibited the proliferation of the HT-1080 cell line (IC_{50} values, 54−1600 nM).

Streptocaulon juventas (Lour.) Merr. is a plant of the Asclepiadaceae family and native in Indochina. In Vietnam,[1] *S. juventas* is called "Ha thu o trang", and its roots are used as a tonic for various conditions such as anemia, chronic malaria, rheumatism, menstrual disorders, neurasthenia, and dyspepsia, as a substitute of "Ha thu o do" (roots of *Polygonum multiflorum* Thunb., Polygonaceae).

In the course of our research on Vietnamese medicinal plants,[2] we previously reported antiproliferative activities of 231 extracts from 77 Vietnamese medicinal plants that have been used as tonics and for treatment of inflammation, cancer, and other conditions.[3] Among them, a MeOH extract of the roots of *S. juventas* showed potent antiproliferative activity against the human HT-1080 fibrosarcoma cell line. To date, there have been no reports on either the constituents or biological activity of this plant. Therefore, we carried out activity-guided separation of the MeOH extract and isolated five new and 11 known cardenolides, two new hemiterpenoids, two known phenylpropanoids, and a known phenylethanoid from an EtOAc- or BuOH-soluble fraction. Their antiproliferative activities were examined against the HT-1080 cell line.

Results and Discussion

Roots of *S. juventas* were extracted with refluxing MeOH, and the MeOH extract was fractionated into Et_2O-, EtOAc-, BuOH-, and H_2O-soluble fractions. Among them, EtOAc- and BuOH-soluble fractions showed potent antiproliferative activities (IC_{50}: 0.57 and 0.19 *μ*g/mL, respectively) against the human HT-1080 fibrosarcoma cell line. Thus, these fractions were subjected to activity-guided separation with silica gel column chromatography, MPLC, and preparative TLC to yield five new (**1**−**5**) and 11 known cardenolides, two new hemiterpenoids (**6**, **7**), two known phenylpropanoids, and a known phenylethanoid.



Compound **1** showed a molecular related ion at *m*/*z* 521.3116 in HRFABMS, corresponding to the molecular formula $C_{29}H_{44}O_8$. The ^1H and ^{13}C NMR (Table 2) data for an ester carbonyl (δ_C 173.8), an olefin (δ_H 5.90; δ_C 116.2,

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Table 1. ^{13}C NMR (100 MHz) Data for **1**−**5**, **9**, and **10**

carbon	**1**[a]	**2**[b]	**3**[c]	**4**[b]	**5**[c]	**9**[d]	**10**[d]
1	71.0	31.0	30.8	31.2	26.1	25.4	25.4
2	31.1	27.5	27.0	27.5	27.3	26.1	26.1
3	73.8	75.8	75.0	74.7	74.0	75.3	75.3
4	29.4	31.3	30.9	31.4	36.5	34.6	34.6
5	30.3	37.5	37.0	38.0	73.2	74.1	73.6
6	25.9	27.8	27.2	27.9	33.2	34.2	34.1
7	20.7	22.5	21.6	22.5	24.7	23.6	23.6
8	41.0	42.7	41.9	42.7	41.0	40.7	40.8
9	36.5	36.9	35.9	36.9	39.2	39.1	39.2
10	39.5	36.3	35.5	36.3	41.2	40.7	40.7
11	20.7	22.3	22.0	22.3	21.9	21.6	21.5
12	38.7	41.0	39.9	41.0	40.0	40.0	40.1
13	49.2	51.0	50.1	51.0	50.0	49.6	49.4
14	83.6	86.4	84.6	86.4	84.7	85.4	85.5
15	32.1	33.4	33.2	33.4	34.0	32.9	33.0
16	26.3	28.0	27.3	28.0	26.7	26.9	26.8
17	50.2	52.2	51.5	52.1	51.3	50.7	50.7
18	15.7	16.4	16.2	16.4	16.2	15.8	15.7
19	18.4	24.1	24.0	24.3	17.2	16.8	16.7
20	176.3	178.3	176.0	178.4	175.9	175.1	174.5
21	73.1	75.3	75.6	75.3	73.7	73.7	73.4
22	116.2	117.8	117.7	117.8	117.7	117.5	117.7
23	173.8	177.2	174.5	177.2	174.5	174.9	174.4
digitoxose (cymarose)							
1	95.5		97.0	97.1		96.5	96.4
2	38.7		37.2	36.4		38.2	33.8
3	66.9		71.8[e]	73.5		67.8	77.3
4	72.6		80.9	84.0		72.7	72.3
5	69.2		69.9	70.3[e]		69.7	70.9
6	18.2		18.7	18.7		18.2	18.2
3-*O*-Ac			170.3				
			21.3				
3-*O*-CH_3				58.2			57.3
digitalose							
1				106.4	101.6		
2				71.6[f]	71.0		
3				85.5	85.7		
4				76.2	76.0		
5				71.4	70.8		
6				17.7	17.6		
3-*O*-CH_3				58.9	58.7		
inner glucose							
1		102.8	105.5	104.8	105.3		
2		75.1[e]	73.8[f]	75.8	76.0		
3		78.1[f]	78.2[g]	77.96[g]	78.3		
4		71.6	71.8[e]	71.7[f]	71.9		
5		77.0	77.3	77.4	78.6		
6		69.7	70.3	70.2[e]	63.1		
outer glucose							
1		104.8	106.4	105.0			
2		75.1[e]	73.7[f]	75.1			
3		77.9[f]	78.1[g]	77.92[g]			
4		71.6	71.7[e]	71.8[f]			
5		77.9[f]	78.4[g]	78.03[g]			
6		62.7	62.8	62.8			

[a−d] Measured in DMSO-d_6, CD_3OD, C_5D_5N, and $CDCl_3$, respectively. [e−g] Assignments may be interchanged in each column.

176.3), an oxymethylene (δ_H 4.96, 4.87; δ_C 73.1), and an anomeric signal (δ_H 4.79; δ_C 95.5), together with the fragment ion at *m*/*z* 391 in FABMS, indicated **1** to be a steroid monoglycoside with a dideoxyhexose as a sugar unit. The dideoxyhexose was concluded to be β-digitoxopyranose (2,6-dideoxy-*ribo*-hexopyranose)[4] on the basis of the ^1H and ^{13}C NMR data (Tables 1 and 2) and analyses of the COSY and HMQC spectra, while the aglycone of **1** was considered to be a cardenolide-type steroid from the absorption of the α,β-unsaturated γ-lactone ring (λ 218 nm; ν 1740, 1620 cm^{-1}) in the UV and IR spectra. By analyses of the COSY, HMQC, and HMBC (Figure 1) spectra, the aglycone was determined to be acovenosigenin A (**8**),[5] which was also isolated from the EtOAc-soluble fraction. The

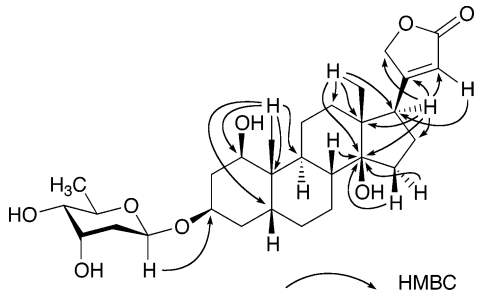

Figure 1. Significant HMBC correlations in **1**.

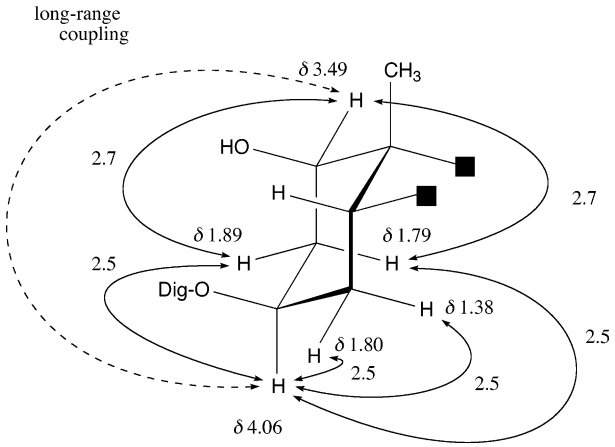

Figure 2. COSY correlations and coupling constants (*J* values in Hz) for ring-A protons of **1** in acetone-d_6.

stereochemistry at C-1 and C-3 was confirmed by analysis of the COSY correlations and coupling constants in acetone-d_6. As can be seen in Figure 2, the coupling constants in acetone-d_6 indicated that ring-A should be in a chair conformation with the hydroxyl groups at C-1 and C-3 in β-axial orientation. The location of the sugar unit was determined to be C-3 on the basis of the glycosylation shifts of C-2 (**1**, δ_C 31.1; **8**, δ_C 32.1), C-3 (**1**, δ_C 73.8; **8**, δ_C 66.8), and C-4 (**1**, δ_C 29.4; **8**, δ_C 33.0) and HMBC correlations between H-1′ of the digitoxose unit and C-3 of the aglycone moiety. From these data, compound **1** was determined as acovenosigenin A 3-*O*-β-digitoxopyranoside.

Compound **2** showed a molecular related ion at *m*/*z* 699.3569 in HRFABMS, corresponding to the molecular formula $C_{35}H_{54}O_{14}$. The ^1H and ^{13}C NMR data (Tables 1 and 2) and fragment ions at *m*/*z* 537 and 357 in FABMS indicated **2** to be a cardenolide disaccharide with two hexoses as the sugar units. Analyses of the COSY, HMQC, and HMBC spectra indicated that the two hexoses are both β-glucopyranose with a (1→6) linkage. The ^{13}C NMR data for **2** indicated that the aglycone is digitoxigenin (**12**),[5] which was isolated from the same extract, and the location of the sugar unit was determined to be at C-3 on the basis of the glycosylation shifts of C-2 (**2**, δ_C 27.5; **12**, δ_C 28.5), C-3 (**2**, δ_C 75.8; **12**, δ_C 67.7), and C-4 (**2**, δ_C 31.3; **12**, δ_C 34.2) along with the HMBC correlation between H-1′ of the glucose unit and C-3 of the aglycone moiety. Thus, **2** was concluded to be digitoxigenin 3-*O*-(6-*O*-β-glucopyranosyl-β-glucopyranoside). This is the first report of the isolation of this compound from natural sources. Synthetic digitoxigenin gentiobioside[6] seemed to have the same structure, but a comparison could not be done because only the spectral data of its acetate were reported.

HRFABMS of compound **3** indicated the molecular formula $C_{43}H_{66}O_{18}$. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **3** resembled those of digitoxigenin 3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-β-digitox-

Table 2. ^{1}H NMR (400 MHz) Data for Sugar Moieties of **1**−**5**, **9**, and **10**

proton	**1**[a]	**2**[b]	**3**[c]	**4**[b]	**5**[c]	**9**[d]	**10**[d]
digitoxose (cymarose)							
1	4.79 dd (9.4, 1.2)		5.14 t (5.8)	4.81 m		4.92 br d (9.3)	4.77 dd (9.9, 2.1)
2	1.80 m		2.42 m	2.08 m		2.07 m	2.23 ddd (14.2, 3.2, 2.1)
	1.53 m		2.42 m	1.57 m		1.70 m	1.60 m
3	3.84 qui (2.7)		6.14 m	3.83 m		4.09 br s	3.63 q (3.2)
4	3.01 ddd (9.3, 6.8, 2.7)		3.84 dd (9.6, 3.1)	3.28 m		3.26 dd (9.2, 2.4)	3.22 dd (9.6, 3.2)
5	3.63 dq (9.3, 6.2)		4.25 m	3.86 m		3.71 dq (9.2, 6.1)	3.57 dq (9.6, 6.0)
6	1.14 d (6.2)		1.62 d (5.5)	1.28 d (6.3)		1.27 d (6.1)	1.27 d (6.0)
3-*O*-Ac			2.05 s				
3-*O*-CH_3				3.41 s			3.43 s
digitalose							
1				4.29 d (7.8)	4.79 d (7.6)		
2				3.65 m	4.42 t (8.8)		
3				3.22 m	3.52 dd (9.6, 2.8)		
4				4.13 d (2.7)	4.33 d (2.7)		
5				3.62 m	3.73 q (6.3)		
6				1.30 d (6.3)	1.56 d (6.3)		
3-*O*-CH_3				3.51 s	3.62 s		
inner glucose							
1		4.31 d (7.8)	5.26 d (7.5)	4.53 d (7.8)	5.14 d (7.6)		
2		3.20 dd (8.8, 7.8)	4.05 m	3.22 m	3.96 t (8.1)		
3		3.35 m	4.34 t (8.0)	3.30 m	4.23 dd (9.2, 8.5)		
4		3.33 m	4.26 m	3.31 m	4.17 dd (9.2, 8.8)		
5		3.43 m	4.05 m	3.45 m	3.94 m		
6		4.10 dd (11.9, 1.6)	4.83 d (9.3)	4.13 dd (11.9, 2.0)	4.56 dd (11.6, 1.8)		
		3.79 dd (11.9, 5.5)	4.25 m	3.77 dd (11.9, 6.2)	4.35 dd (11.6, 5.6)		
outer glucose							
1		4.39 d (7.8)	4.83 d (8.3)	4.39 d (7.8)			
2		3.20 dd (8.8, 7.8)	3.95 m	3.18 m			
3		3.35 m	4.11 m	3.32 m			
4		3.30 m	3.90 m	3.30 m			
5		3.26 m	4.09 m	3.25 m			
6		3.86 dd (12.0, 2.0)	4.53 dd (11.9, 2.1)	3.86 m			
		3.66 dd (12.0, 5.2)	4.34 dd (11.9, 5.1)	3.66 m			

[a−d] Measured in DMSO-d_6, CD_3OD, C_5D_5N, and $CDCl_3$, respectively.

opyranoside] (**13**)[7] isolated from the same extract, but they revealed the presence of an additional acetyl group (δ_H 2.05; δ_C 170.3, 21.3). The acetyl group was located at C-3′ of the digitoxose unit on the basis of the HMBC correlation between the carbonyl carbon of the acetyl group and H-3′ of the digitoxose unit. Thus, **3** was concluded to be digitoxigenin 3-*O*-[*O*-*β*-glucopyranosyl-(1→6)-*O*-*β*-glucopyranosyl-(1→4)-3-*O*-acetyl-*β*-digitoxopyranoside].

Compound **4** showed a molecular related ion at *m*/*z* 1025.4965 in HRFABMS, corresponding to the molecular formula $C_{49}H_{78}O_{21}$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) indicated **4** to be a digitoxigenin tetraglycoside. The sugar unit was determined to consist of a *β*-cymaropyranose, a *β*-digitalopyranose, and two *β*-glucopyranoses by analyses of the COSY and HMQC spectra. The HMBC spectrum of **4** showed correlations between H-1′′′′ of the outer glucose unit and C-6′′′ of the inner glucose unit, between H-1′′′ of the inner glucose unit and C-4′′ of the digitalose unit, between H-1′′ of the digitalose unit and C-4′ of the cymarose unit, and between H-1′ of the cymarose unit and C-3 of the aglycone moiety. Thus, **4** was determined as digitoxigenin 3-*O*-[*O*-*β*-glucopyranosyl-(1→6)-*O*-*β*-glucopyranosyl-(1→4)-*O*-*β*-digitalopyranosyl-(1→4)-*β*-cymaropyranoside].

Compound **5** showed a molecular related ion at *m*/*z* 735.3613 in HRFABMS, corresponding to the molecular formula $C_{36}H_{56}O_{14}$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) resembled those of **16**[8] and **17**[9] isolated from the same extract, indicating **5** to be a periplogenin diglycoside. By analyses of the COSY and HMQC spectra, the sugars were determined to be a *β*-digitalopyranose and a *β*-glucopyranose, and the HMBC correlations between H-1′′ of the glucose unit and C-4′ of the digitalose unit, between H-4′ of the digitalose unit and C-1′′ of the glucose unit, and between H-1′ of the digitalose unit and C-3 of the aglycone moiety confirmed the connectivity of the sugar units. Thus, **5** was concluded to be periplogenin 3-*O*-(4-*O*-*β*-glucopyranosyl-*β*-digitalopyranoside). The isomers of **5**, periplogenin 3-*O*-(4-*O*-glucosyldigitaloside) and emicin, were reported as constituents of *Strophanthus preussii*[10] and differ markedly from **5** in their specific rotations.

HRFABMS of compounds **6** and **7** indicated the molecular formulas $C_{20}H_{38}O_{11}$ and $C_{19}H_{36}O_{10}$, respectively. Fragment ions at *m*/*z* 309 and 147 in the FABMS and the presence of two anomeric signals in the ^{1}H and ^{13}C NMR spectra of **6** (Table 3) indicated it to be a diglycoside, whose sugar units consist of a *β*-glucopyranose and an α-rhamnopyranose. The HMBC correlations between H-6′ of the

Table 3. ^{1}H and ^{13}C NMR Data for **6** and **7** (in CD_3OD)

	6		**7**	
position	δ_H	δ_C	δ_H	δ_C
1	3.89 m	71.1	3.91 ddd (9.5, 7.8, 5.5)	69.7
	3.58 m		3.53 dt (9.5, 7.7)	
2	1.61 m	27.4	1.73 m	36.4
3	1.27 m	48.6	1.59 m	33.3
4	3.83 m	69.5	0.89 d (6.6)	20.5
5	1.14 d (6.4)	20.0	1.58 m	35.1
			1.39 m	
6	1.85 m	29.4	0.84 d (6.8)	15.7
7	0.92 d (6.8)	21.8	0.83 d (6.9)	18.4
8	0.91 d (6.6)	19.4		
glucose				
1′	4.24 d (7.8)	104.2	4.21 d (7.8)	104.5
2′	3.16 dd (9.0, 7.8)	75.0	3.14 dd (9.1, 7.8)	75.1
3′	3.33 m	78.0	3.32 m	78.1
4′	3.25 m	71.6	3.25 t (8.8)	71.7
5′	3.40 m	76.8	3.38 m	76.8
6′	3.96 dd (11.2, 1,4)	68.2	3.96 dd (11.2, 1.7)	68.2
	3.60 m		3.60 dd (11.2, 6.0)	
rhamnose				
1″	4.74 d (1.7)	102.2	4.73 d (1.6)	102.3
2″	3.83 m	72.3	3.82 dd (3.3, 1.6)	72.4
3″	3.66 m	72.1	3.65 m	72.2
4″	3.35 m	74.0	3.33 m	74.0
5″	3.66 m	69.7	3.66 m	69.8
6″	1.26 d (6.1)	18.1	1.25 d (6.4)	18.1

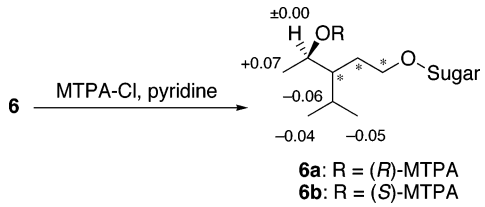

Figure 3. $\Delta\delta$ ($= \delta^R - \delta^S$) values obtained from the MTPA esters **6a** and **6b** in $CDCl_3$. *$\Delta\delta$ values for these protons could not be calculated due to signal ovarlapping.

glucose unit and C-1″ of the rhamnose unit, between H-1″ of the rhamnose unit and C-6′ of the glucose unit, between H-1′ of the glucose unit and C-1 of the aglycone moiety, and between H-1 of the aglycone moiety and C-1′ of the glucose unit confirmed the presence of a β-rutinose (6-*O*-α-rhamnopyranosyl-β-glucopyranose) unit at C-1. The ^{1}H and ^{13}C NMR data of **7** and GC analysis of chiral derivatives of sugars[11] in an acid hydrolysate showed **7** to be a β-rutinoside. The aglycone of **6** was determined as 3-isopropylpentane-1,4-diol by analysis of the COSY spectrum. The absolute configuration at C-4 was determined as *R* by the advanced Mosher's method (Figure 3),[12] but that at C-3 could not be determined. On the other hand, the aglycone of **7** was determined as (*R*)-2-ethyl-3-methyl-1-butanol on the basis of specific rotation of an acid hydrolysate ($[\alpha]^{21}_D$ +10.5°; $[\alpha]_D$ of (*S*)-(−)-2-ethyl-3-methyl-1-butanol[15] −9.2°). Thus, **6** and **7** were concluded to be (3ξ,4*R*)-4-hydroxy-3-isopropylpentyl β-rutinoside and (*R*)-2-ethyl-3-methylbutyl β-rutinoside, respectively.

The known compounds **9** and **10** were identified as periplogenin 3-*O*-β-digitoxoside[14] and periplogenin 3-*O*-β-cymaroside (periplocymarin)[15] by analyses of COSY, HMQC, and HMBC spectra, respectively, although the published data, which included only UV, IR, and optical rotation data, were not enough for satisfactory comparison. The other known compounds were identified by spectroscopic analyses and comparisons with published data as acovenosigenin A (**8**),[5] periplogenin (**11**),[5,8] digitoxigenin (**12**),[5] digitoxigenin 3-*O*-[*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-β-D-digitoxopyranoside] (**13**),[6] digitoxigenin sophoroside (**14**),[16] echujin (**15**),[17] periplogenin glucoside (**16**),[8] corchorusoside C (**17**),[9] subalpinoside (**18**),[18] caffeic acid (**19**),[19] 4,5-di-*O*-caffeoylquinic acid (**20**),[20] and 2-phenylethyl rutinoside (**21**),[21] respectively. This is the first report of the isolation of compounds **8**, **9**, **13**−**15**, **17**, **18**, and **21** from Asclepiadaceae plants and the first isolation of compound **14** from wild plants.

Table 4. Antiproliferative Activities of Cardenolides Isolated from *Streptocaulon juventas* toward Human HT-1080 Fibrosarcoma Cells

compound	IC_{50} (nM)
1	1100
2	1600
3	590
4	220
5	670
8	1200
9	93
10	96
11	810
12	1500
13	54
14	460
15	55
16	160
17	92
18	180
5-fluorouracil	3700
doxorubicin	59

The isolated compounds were examined for their antiproliferative activity toward the human HT-1080 fibrosarcoma cell line (Table 4). Cardenolides **1**−**16** exhibited more potent activities (IC_{50}, 54−1600 nM) than a positive control, 5-fluorouracil (IC_{50}, 3700 nM), and **13** and **15** (IC_{50}, 54 and 55 nM, respectively) were as potent as doxorubicin (IC_{50}, 59 nM). It appears that glycosylation on the 3-hydroxyl group may increase the activity, with the triglycoside being especially potent.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. UV and IR spectra were obtained on a Shimadzu UV-160A UV−visible spectrometer and a Shimadzu IR-408 infrared spectrometer, respectively. NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard. FABMS and HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as a matrix. Column chromatography was performed with silica gel 60 (70−230 mesh; Nacalai Tesque, Inc., Kyoto, Japan) or silica gel 60 N (spherical, neutral; Kanto Chemical Co., Inc., Tokyo, Japan), and analytical and preparative TLC were carried out on precoated silica gel 60 F_{254} plates (0.25 or 0.5 mm thickness) or RP-18 F_{254S} (0.25 mm thickness) (both Merck, Darmstadt, Germany). Medium-pressure liquid chromatography (MPLC) was performed with Chemco low-prep pump model 81-M-2 on a Lobar column (LiChroprep RP-18, 310 mm × 25 mm i.d.).

Plant Material. Roots of *Streptocaulon juventas* (Lour.) Merr. (Asclepiadaceae) were collected at Seven-Mountain area, Tinh Bien Village, Chan Phu District, Angiang Province, Vietnam, in June 2000. They were identified by Prof. Le Cong Kiet (Department of Botany, National University-Hochiminh City, Hochiminh, Vietnam), and the voucher sample (TMPW 21639) is preserved at the Museum of Materia Medica, Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation. Air-dried roots of *S. juventas* (2.9 kg) were cut into small pieces and extracted with MeOH (12 L, reflux, 3 h, × 3). The MeOH extract was evaporated

under reduced pressure to give a MeOH extract (320 g; IC_{50} value toward HT-1080 cell line, 2.9 μg/mL). The extract was partitioned between Et_2O-H_2O, EtOAc−H_2O, and BuOH−H_2O, successively, to yield Et_2O (63.8 g; IC_{50}, 5.3 μg/mL), EtOAc (10.6 g; IC_{50}, 0.57 μg/mL), BuOH (23.3 g; IC_{50}, 0.19 μg/mL), and H_2O (210 g; IC_{50}, 79 μg/mL) fractions.

The EtOAc fraction (4.7 g) was subjected to silica gel column chromatography with a MeOH−$CHCl_3$ gradient system to yield 22 fractions [fractions 1−4 ($CHCl_3$ eluates): 50 mg, 71 mg, 76 mg, 74 mg; fractions 5−9 (1% MeOH eluates): 20 mg, 66 mg, 54 mg, 28 mg, 140 mg; fractions 10 and 11 (2% MeOH eluates): 70 mg, 154 mg; fractions 12−16 (5% MeOH eluates): 238 mg, 338 mg, 411 mg, 108 mg, 211 mg; fractions 17 and 18 (10% MeOH eluates): 713 mg, 239 mg; fractions 19 and 20 (15% MeOH eluates): 199 mg, 582 mg; fraction 21 (50% MeOH eluate): 71 mg; fraction 22 (MeOH eluate): 183 mg]. These fractions were examined for antiproliferative activity (Figure S1 in Supporting Information), and fractions 11−20, which showed potent activities, were further separated.

Fractions 11 (IC_{50}, 3.1 μg/mL) and 12 (IC_{50}, 2.9 μg/mL) were combined, and a portion of the pooled fraction (95.5 mg) was subjected to reversed-phase preparative TLC (MeCN−H_2O, 2:3) to yield digitoxigenin (**12**, 4.6 mg).[5]

Fraction 13 (327 mg; IC_{50}, 0.20 μg/mL) was rechromatographed with silica gel to afford 16 subfractions. Subfraction 6 gave periplocymarin (**10**, 26.6 mg),[15] while normal-phase preparative TLC of subfraction 7 ($CHCl_3$−Me_2CO, 2:1) yielded acovenosigenin A (**8**, 2.8 mg).[5]

Fraction 14 (101 mg; IC_{50}, 2.1 μg/mL) was subjected to reversed-phase preparative TLC (MeCN−H_2O, 2:3) to yield acovenosigenin A 3-*O*-β-digitoxopyranoside (**1**, 2.9 mg), periplogenin (**12**, 8.2 mg),[5,8] and caffeic acid (**19**, 3.5 mg).[19]

Fraction 15 (98.8 mg; IC_{50}, 0.16 μg/mL) was further separated by silica gel column chromatography, followed by normal-phase preparative TLC ($CHCl_3$−Me_2CO, 1:1), to yield **1** (5.0 mg).

Fraction 17 (693 mg; IC_{50}, 0.15 μg/mL) was rechromatographed over silica gel to afford 11 subfractions. Subfraction 5 gave periplogenin digitoxoside (**9**, 110 mg).[14] Subfractions 4 and 6 were purified by normal-phase preparative TLC ($CHCl_3$−Me_2CO, 1:1) to yield **11** (1.2 mg) and **9** (6.5 mg), respectively.

Fraction 19 (102 mg; IC_{50}, 0.75 μg/mL) was subjected to reversed-phase preparative TLC (MeCN−H_2O, 2:3) to yield subalpinoside (**18**, 5.1 mg),[18] while reversed-phase preparative TLC (MeCN−H_2O, 1:2) of fraction 20 (117 mg; IC_{50}, 1.7 μg/mL) yielded 4,5-di-*O*-caffeoylquinic acid (**20**, 8.6 mg).[20]

The BuOH fraction (13.2 g) was subjected to silica gel column chromatography with a MeOH−$CHCl_3$ gradient system to yield 21 fractions [fractions 1−4 (5% MeOH eluates): 195 mg, 223 mg, 217 mg, 459 mg; fractions 5−7 (10% MeOH eluates): 188 mg, 847 mg, 2.07 g; fractions 8 and 9 (15% MeOH eluates): 681 mg, 1.59 g; fractions 10−14 (20% MeOH eluates): 614 mg, 1.24 g, 779 mg, 487 mg, 242 mg; fractions 15 and 16 (30% MeOH eluates): 800 mg, 763 mg; fractions 17 and 18 (40% MeOH eluates): 426 mg, 341 mg, 212 mg; fractions 20 and 21 (MeOH eluates): 399 mg, 131 mg]. These fractions were examined for antiproliferative activity (Figure S1 in Supporting Information), and fractions 10−12, which showed potent activities, were further separated.

Fraction 10 (500 mg; IC_{50}, 66 ng/mL) was subjected to MPLC with a MeCN−H_2O gradient system (20−50% MeCN) to yield 15 subfractions. Subfractions 2, 5, and 11 gave (*R*)-2-ethyl-3-methylbutyl β-rutinoside (**7**, 51.0 mg), digitoxigenin sophoroside (**14**, 9.9 mg),[16] and echujin (**15**, 87.4 mg),[17] respectively. Subfractions 4 (47.9 mg) and 7 (24.4 mg) were purified by reversed-phase preparative TLC (MeCN−H_2O, 3:7 and 1:2, respectively) to yield **14** (2.7 mg) and periplogenin 3-*O*-(4-*O*-β-glucopyranosyl-β-digitalopyranoside) (**5**, 3.8 mg), respectively. Subfraction 10 (32.9 mg) was subjected to reversed-phase preparative TLC (MeCN−H_2O, 1:2) to yield digitoxigenin 3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-β-digitoxopyranoside] (**13**, 7.0 mg)[6] and **15** (2.6 mg). Reversed-phase preparative TLC (MeCN−H_2O, 7:13) of subfraction 12 yielded digitoxigenin 3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-3-*O*-acetyl-β-digitoxopyranoside] (**3**, 10.8 mg) and digitoxigenin 3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-*O*-β-digitalopyranosyl-(1→4)-β-cymaropyranoside] (**4**, 14.1 mg).

Fraction 11 (990 mg; IC_{50}, 69 ng/mL) was subjected to MPLC with a MeCN−H_2O gradient system (20−50% MeCN) to yield eight subfractions. Subfractions 3 and 5 gave (4*R*)-4-hydroxy-3-isopropylpentyl β-rutinoside (**6**, 5.8 mg) and corchorusoside C (**17**, 145 mg),[9] respectively. Subfraction 2 (101 mg) was purified by reversed-phase preparative TLC (MeCN−H_2O, 3:7) to yield 2-phenylethyl rutinoside (**21**, 12.9 mg).[21] Reversed-phase preparative TLC (MeCN−H_2O, 3:7) of subfraction 4 (113 mg) yielded **5** (7.1 mg), **6** (8.0 mg), periplogenin glucoside (**16**, 18.4 mg),[8] and **17** (3.5 mg).

Fraction 12 (675 mg; IC_{50}, 68 ng/mL) was also separated by MPLC with a MeCN−H_2O gradient system (20−50% MeCN) to yield 10 subfractions. Subfraction 4 gave periplogenin 3-*O*-β-genitiobioside (**2**, 13.3 mg),[6] while subfractions 3 (41.7 mg) and 5 (47.4 mg) were purified by reversed-phase preparative TLC (MeCN−H_2O, 1:4 and 3:7, respectively) to yield **2** (6.6 mg) and **14** (17.4 mg), respectively. Subfraction 7 (130 mg) was also subjected to reversed-phase preparative TLC (MeCN−H_2O, 3:17) to yield **13** (14.6 mg) and **15** (18.9 mg). Reversed-phase preparative TLC (MeCN−H_2O, 2:3) of subfraction 8 yielded **4** (32.7 mg) and **12** (2.5 mg).

Acovenosigenin A 3-*O*-β-digitoxoside (1): colorless crystals (acetone); mp 117−121 °C; $[\alpha]^{23}_D$ −1.0° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.1) nm; IR ($CHCl_3$) ν_{max} 3450, 1740, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.90 (1H, t, J = 1.7 Hz, H-22), 4.96 (1H, br d, J = 18.1 Hz, H-21), 4.87 (1H, dd, J = 18.1, 1.5 Hz, H-21), 4.07 (1H, br s, H-3), 3.51 (1H, br s, H-1), 2.71 (1H, m, H-17), 0.94 (3H, s, H_3-19), 0.77 (3H, s, H_3-18), and Table 2; ^{13}C NMR (DMSO-d_6), see Table 1; FABMS *m/z* 521 $[M + H]^+$, 391, 373; HRFABMS *m/z* 521.3116 (calcd for $C_{29}H_{45}O_8$ $[M + H]^+$, 521.3114).

Digitoxigenin 3-*O*-β-genitiobioside (2): light brown amorphous solid; $[\alpha]^{21}_D$ −17.1° (*c* 0.8, MeOH); ^1H NMR (CD_3OD) δ 5.89 (1H, br s, H-22), 5.03 (1H, dd, J = 18.3, 1.4 Hz, H-21), 4.90 (1H, dd, J = 18.3, 1.8 Hz, H-21), 4.07 (1H, br s, H-3), 2.83 (1H, m, H-17), 0.96 (3H, s, H_3-19), 0.88 (3H, s, H_3-18), and Table 2; ^{13}C NMR (CD_3OD), see Table 1; FABMS *m/z* 721 $[M + Na]^+$, 699 $[M + H]^+$, 681 $[M + H - H_2O]^+$, 537, 519, 357; HRFABMS *m/z* 699.3569 (calcd for $C_{35}H_{55}O_{14}$ $[M + H]^+$, 699.3592).

Digitoxigenin 3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-3-*O*-acetyl-β-digitoxopyranoside] (3): light brown amorphous solid; $[\alpha]^{21}_D$ −5.5° (*c* 0.6, MeOH); ^1H NMR (C_5D_5N) δ 6.13 (1H, br s, H-22), 5.32 (1H, d, J = 17.9 Hz, H-21), 5.04 (1H, d, J = 17.9 Hz, H-21), 4.23 (1H, m, H-3), 2.79 (1H, m, H-17), 1.02 (3H, s, H_3-19), 0.88 (3H, s, H_3-18), and Table 2; ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* 893 $[M + Na]^+$; HRFABMS *m/z* 893.4110 (calcd for $C_{43}H_{66}O_{18}Na$ $[M + Na]^+$, 893.4147).

Digitoxigenin 3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-*O*-β-digitalopyranosyl-(1→4)-β-cymaropyranoside] (4): light brown amorphous solid; $[\alpha]^{23}_D$ −9.5° (*c* 0.3, MeOH); ^1H NMR (CD_3OD) δ 5.88 (1H, br t, J = 1.6 Hz, H-22), 5.02 (1H, dd, J = 18.4, 1.6 Hz, H-21), 4.90 (1H, dd, J = 18.4, 1.6 Hz, H-21), 3.98 (1H, br s, H-3), 2.82 (1H, m, H-17), 0.93 (3H, s, H_3-19), 0.87 (3H, s, H_3-18), and Table 2; ^{13}C NMR (CD_3OD), see Table 1; FABMS *m/z* 1025 $[M + Na]^+$, 519, 375, 357, 339; HRFABMS *m/z* 1025.4965 (calcd for $C_{49}H_{78}O_{21}Na$ $[M + Na]^+$, 1025.4933).

Periplogenin 3-*O*-(4-*O*-β-glucopyranosyl-β-digitalopyranoside) (5): light brown amorphous solid; $[\alpha]^{22}_D$ −9.8° (*c* 0.3, pyridine); ^1H NMR (C_5D_5N) δ 6.14 (1H, br s, H-22), 5.31 (1H, dd, J = 18.2, 1.5 Hz, H-21), 5.04 (1H, br d, J = 18.2 Hz, H-21), 4.47 (1H, br s, H-3), 2.81 (1H, m, H-17), 1.06 (3H, s, H_3-19), 1.03 (3H, s, H_3-18), and Table 2; ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* 735 $[M + Na]^+$, 391, 373, 355, 337; HRFABMS *m/z* 735.3613 (calcd for $C_{36}H_{56}O_{14}Na$ $[M + Na]^+$, 735.3568).

(4*R*)-4-Hydroxy-3-isopropylpentyl β-rutinoside (6): light brown amorphous solid; $[\alpha]^{21}_D$ −28.4° (*c* 0.5, MeOH); ^1H and ^{13}C NMR, see Table 3; FABMS *m/z* 493 $[M + K]^+$, 477 [M +

Na]$^+$, 455 [M + H]$^+$, 309, 293, 279, 147, 129; HRFABMS *m/z* 455.2456 (calcd for $C_{20}H_{39}O_{11}$ [M + H]$^+$, 455.2492).

(*R*)-2-Ethyl-3-methylbutyl β-rutinoside (7): light brown amorphous solid; $[\alpha]^{23}_D$ −35.5° (*c* 0.4, MeOH); ^{1}H and ^{13}C NMR, see Table 3; FABMS *m/z* 469 [M + 2Na − H]$^+$, 447 [M + Na]$^+$, 425 [M + H]$^+$, 309, 293, 279; HRFABMS *m/z* 425.2379 (calcd for $C_{19}H_{37}O_{10}$ [M + H]$^+$, 425.2387).

Periplogenin digitoxoside (9): light brown amorphous solid; $[\alpha]^{23}_D$ +17.1° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.87 (1H, br s, H-22), 5.00 (1H, d, *J* = 18.2 Hz, H-21), 4.82 (1H, d, *J* = 18.2 Hz, H-21), 4.16 (1H, br s, H-3), 2.78 (1H, dd, *J* = 8.8, 5,6 Hz, H-17), 0.92 (3H, s, H_3-19), 0.87 (3H, s, H_3-18), and Table 2; ^{13}C NMR ($CDCl_3$), see Table 1; FABMS *m/z* 543 [M + Na]$^+$, 521 [M + H]$^+$, 503 [M + H − H_2O]$^+$, 391, 373, 355, 337; HRFABMS *m/z* 521.3153 (calcd for $C_{29}H_{45}O_8$ [M + H]$^+$, 521.3114).

Periplocymarin (10): light brown amorphous solid; $[\alpha]^{23}_D$ +31.4° (*c* 0.4, EtOH); UV (EtOH) λ_{max} (log ε) 215 (4.1) nm; ^{1}H NMR ($CDCl_3$) δ 5.88 (1H, br s, H-22), 4.98 (1H, dd, *J* = 18.2, 1.3 Hz, H-21), 4.81 (1H, dd, *J* = 18.2, 1.8 Hz, H-21), 4.15 (1H, br s, H-3), 2.79 (1H, dd, *J* = 9.3, 5.6 Hz, H-17), 2.02 (1H, dd, *J* = 15.4, 2.9 Hz, H-4), 0.94 (3H, s, H_3-19), 0.88 (3H, s, H_3-18), and Table 2; ^{13}C NMR ($CDCl_3$), see Table 1; FABMS *m/z* 557 [M + Na]$^+$, 535 [M + H]$^+$, 517 [M + H − H_2O]$^+$, 391, 373, 355, 337; HRFABMS *m/z* 535.3242 (calcd for $C_{30}H_{47}O_8$ [M + H]$^+$, 535.3271).

MTPA Esterification of 6. To a stirred solution of **6** (1.1 mg) in pyridine (0.5 mL) was added (*S*)-(+)-MTPA chloride or (*R*)-(−)-MTPA chloride (10 μL), and the mixture was stirred overnight at room temperature. To the reaction mixture was added water, and the solution was extracted with EtOAc. The organic layer was washed with saturated $CuSO_4$ solution and saturated $NaHCO_3$ solution, successively, and then subjected to normal-phase preparative TLC with hexane−EtOAc (3:2) to afford **6a** (0.85 mg) or **6b** (0.54 mg).

(*R*)-MTPA ester 6a: colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 5.20 (1H, m, H-4), 1.74 (1H, m, H-6), 1.28 (3H, m, H_3-5), 0.83 (3H, m, H_3-7), 0.72 (3H, d, *J* = 6.8 Hz, H_3-8); FABMS *m/z* 1317 [M − H]$^-$.

(*S*)-MTPA ester 6b: colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 5.20 (1H, m, H-4), 1.80 (1H, m, H-6), 1.21 (3H, m, H_3-5), 0.88 (3H, m, H_3-7), 0.76 (3H, d, *J* = 6.8 Hz, H_3-8); FABMS *m/z* 1317 [M − H]$^-$.

Acid Hydrolysis and Sugar Analysis.[11] A solution of **7** (5.9 mg) in 1 N HCl (dioxane−H_2O, 1:1; 1.5 mL) was heated at 85 °C for 4 h. After cooling, water was added, and the solution was extracted with EtOAc three times. The organic layer was combined and dried to yield (*R*)-2-ethyl-3-methylbutanol (1.5 mg). The aqueous layer was neutralized with Amberlite IRA67 (OH^-), and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in pyridine (0.1 mL), and 0.1 M L-cysteine methyl ester hydrochloride in pyridine (0.1 mL) was added. After the mixture was heated at 60 °C for 2 h, trimethylsilylimidazole (0.1 mL) was added, and the mixture was heated at 60 °C for 1 h. The reaction mixture was partitioned between hexane and water (each 0.1 mL), and the organic layer was analyzed on a Hewlett-Packard HP6890 series GC system; column, DB-5MS 30 m × 0.32 mm (J&W Scientific Inc.); column temperature, 210 °C; detector temperature, 270 °C; injection temperature, 270 °C. Standard sugars gave peaks at t_R (min) 13.29 and 14.17 for D- and L-glucose and 8.65 for L-rhamnose, respectively. Although we could not obtain standard D-rhamnose commercially, it was reported that derivatives of L- and d-rhamnose were well separated from each other as those of other sugars.[11]

Cells. A highly metastatic human HT-1080 fibrosarcoma cell line[22] (ATCC # CCL-121) was obtained from American Type Culture Collection (Rockville, MD). The HT-1080 cell line was maintained in 75 cm^2 cell culture flasks (Corning, NY) in Eagle's minimum essential (EME) medium (Nissui Pharmaceutical Co., Ltd, Tokyo) supplemented with 10% heat-inactivated fetal calf serum (Invitrogen Co., Carlsbad, CA) and 2 mM L-(+)-glutamine (Wako Pure Chemical Industries Ltd., Osaka, Japan) and pH-balanced with 10% $NaHCO_3$ at 37 °C under humidified 5% CO_2.

Antiproliferative Activity. Viability of HT-1080 cells in the presence or absence of experimental fractions or compounds was determined using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay[23] as described previously.[3] In brief, exponentially growing cells were harvested, and 100 μL of medium with 2 × 10^3 cells suspended was plated in each well in a 96-well plate. After 24 h incubation at 37 °C under humidified 5% CO_2 to allow cell attachment, the cells were treated with varying concentrations of test specimens in their respective medium (100 μL) and incubated for 72 h under the same conditions. After 2 h of the MTT (0.4−0.5 mg/mL, 100 μL; Aldrich Chemical Co., Inc., Milwaukee, WI) addition, the formazan formed was extracted with DMSO and its amount was measured spectrophotometrically at 550 nm with a Perkin-Elmer HTS-7000 bio assay reader (Norwalk, CT).

Each sample was dissolved in DMSO (25 μL per 1.0 mg of sample), and Dulbecco's PBS (Nissui Pharmaceutical Co., Ltd.; 975 μL per 1.0 mg of sample) was added to make a stock solution. Each solution was diluted with the medium when the final concentration of DMSO was less than 0.25%. 5-Fluorouracil (Tokyo Kasei Kogyo Co., Ltd., Tokyo) and doxorubicin (Kyowa Hakko Kogyo Co., Ltd., Tokyo) were used as positive controls. Cellular viabillity and IC_{50} values were calculated from the mean values of data from four wells. Cellular viability (%) = [Abs(test sample)/Abs(control)] × 100.

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Supporting Information Available: Figure showing antiproliferative activity of the fractions of *Streptocaulon juventas*. This information is available free of charge via the Internet at http://pubs.acs.org.

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